

Is the archeopyle of *Tuberculodinium vancampoeae* (Rossignol) (Gonyaulacales, Dinophyceae) on the hypocyst ?

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Abstract. The archeopyle type in modern dinoflagellate cysts is classified into the following saphopylic, theropylic and cryptopylic types. Within the saphopylic type, *Tuberculodinium vancampoeae* (=cyst of *Pyrophacus steinii*) is the only species generally accepted as having an archeopyle developed on the hypocyst. However, the archeopyle type of this species has been alternatively explained as being precingular rather than hypocystal. New observations have led us to conclude that the archeopyle of *T. vancampoeae* is neither hypocystal nor precingular, but epicystal in type. Specimens of the hypnozygote of *P. steinii* recovered in a plankton sample from Omura Bay, West Japan show archeopyle sutures formed neither on antapical nor precingular sides but on the apical side where they are topologically related to the distribution of large and barrel-shaped processes and remaining thecal plates that overlap the hypnozygote. The hypnozygotes of *P. steinii*, wrapped in their planozygotic thecae, were carefully observed at germination during an incubation experiment. One of the living hypnozygotes was still enveloped by the thecae of the planozygote. Then, several weeks after the encystment, a motile cell germinated from the hypnozygote through one opening (= archeopyle) formed on one flat side. Since the thecae of the planozygote was still attached to the surface of the hypnozygote, the position of the germination site was easily determined with relation to the thecal plates. Above the archeopyle consisting of two paraplates, the apical pore plate with two furrows was observed. It is clear that the archeopyle was formed not on the antapical but on the apical side.

Key words : Archeopyle, dinoflagellate cyst, Dinophyceae, hypnozygote, planozygote, *Pyrophacus*, *Tuberculodinium*

Introduction

For the identification of both modern and fossil dinoflagellate cysts, the archeopyle is one of the important characteristics (e.g., Evitt, 1963, 1985). The archeopyle type in modern dinoflagellate cysts is classified into saphopylic, theropylic and cryptopylic types in the relation to the plate series for the armored group and position for the unarmored group (Matsuoka, 1988). The saphopylic archeopyle type is developed in most gonyaulacoid and protoperidinioid cysts except for an unclear type observed in the cysts of *Alexandrium* species and *Gonyaulax verior*. The other modern cysts in these groups are apical, intercalary, precingular, epicystal or some combinations thereof. Only one example of a hypocystal archeopyle has been generally accepted, this being *Tuberculodinium vancampoeae* (Rossignol) (=cyst of *Pyrophacus steinii steinii* and *P. steinii vancampoeae*) by Wall and Dale (1971) and followed by Matsuoka (1985).

A hypocystal archeopyle in fossil dinoflagellates has been

proposed in the cyst genus *Caligodinium* by Manum and Williams (1995) based on the careful observation and more rational interpretation of the topological relation of each paraplate. Another example of a hypocystal archeopyle (probably involving loss of postcingular paraplates) archeopyle was introduced for siliceous fossils of some species of *Peridinites* (=a synonym of *Lithoperidinium* according to Lentin and Williams, 1993), *Peridinites oamaruensis* and *P. parvalus*, with semidiagrammatic illustrations by Dale (1978). However, Harding and Lewis (1994) concluded that fossil *Peridinites* is not a cyst but a silicified theca and therefore the opening observed on the hypotheca is not a real germination apparatus (= archeopyle). For the moment, the hypocystal archeopyle seems to be only a hypothetical interpretation for *Peridinites*.

Following Wall and Dale's (1971) proposal for a hypocystal archeopyle in *P. steinii* cysts, there have been no effective and persuasive studies on the hypocystal archeopyle type in modern dinoflagellates. We will prove that the archeopyle

of *T. vancampoe* is not hypocystal but epicystal on the basis of observations for plankton and incubated specimens of *P. steinii*.

**Previous study on the archeopyle type of
*Tuberculodinium vancampoe***

The fossil cyst of *Pyrophacus steinii* (= *Tuberculodinium vancampoe*) was first described from the Pleistocene of Israel as a new species questionably assigned to *Pterosper-*

mopsis, *P.?* *vancampoe* by Rossignol (1962). Later this species was transferred to *Tuberculodinium*, then a newly erected dinophycean genus by Wall (1967). In the emended diagnosis for *Tuberculodinium vancampoe*, Wall (1967) mentioned that the archeopyle of this species is a large compound type consisting of precingular and anterior intercalary paraplates. Drugg (1970) also interpreted the archeopyle of fossil *Tuberculodinium* as precingular.

Then in 1971, Wall and Dale concluded that the archeopyle of this cyst is not compound precingular but hypocystal

Table 1.

	Wall (1967)	Drugg (1970)	Wall & Dale (1971)	Stover & Evitt (1978)	Wrenn & Damassa (1989)	This study
Material	fossil cysts	fossil cysts	living hypanozygotes incubated	literature	fossil cysts	living vegetative cells, planozygotes and hypanozygotes
Compression direction in cysts	dorso-ventrally	dorso-ventrally	anterio-posterior	anterio-posterior	dorso-ventral cyst rotated 90 degree within the planozygose	anterio-posterior
Archeopyle type	compound ; precingular + anterior intercalary	precingular	hypocystal	compound ; antapical	precingular	compound ; epicystal
Wall features	not mentioned	not intratabular	not mentioned	intratabular	not mentioned	?

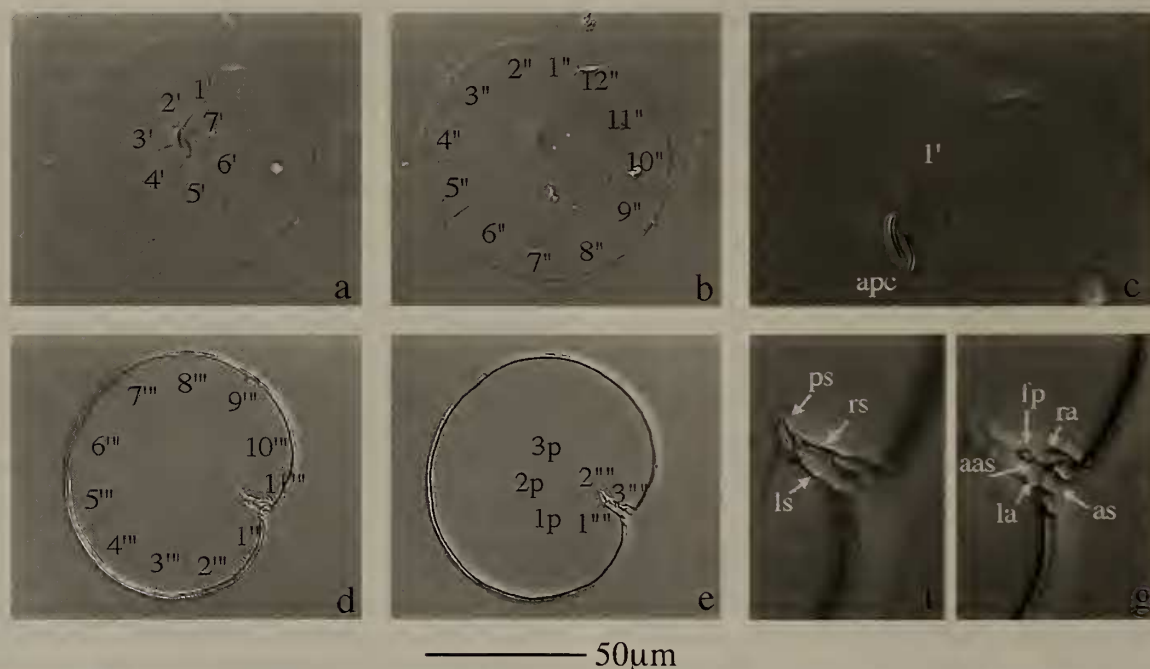


Figure 1. Plate tabulation in vegetative cell. **a**; apical plate series, **b**; precingular plate series, **c**; apical pore complex (apc) and 1', **d**; postcingular plate series, **e**; posterior intercalary and antapical plate series, **f** and **g**; sulcal plates, as: anterior sulcal plate, ra: right accessory sulcal plate, la: left accessory sulcal plate, aas: anterior accessory sulcal plate, rs: right sulcal plate, ls: left sulcal plate, ps: posterior sulcal plate, fp: flagellar pore.

based on the result of cyst incubation experiments on *P. steinii*. This explanation was followed by Matsuoka (1985) based also on the incubation method. Stover and Evitt (1978) suggested that *Tuberculodinium* has an antapical rather than hypocystal archeopyle. However, the possibility that the archeopyle type of *Tuberculodinium* is not hypocystal but precingular was raised by Wrenn and Damassa (1989).

Thus, the archeopyle type of *T. vancampoe* remains unclear and the subject of discussion (see Table 1).

Material and method

A plankton sample was collected with a net haul in June of 1983 from Omura Bay, West Japan, and preserved with 5% formalin. Several specimens of the hypnozygote of *P. steinii*

were picked up with a micropipette and then mounted on a slide glass with glycerine jelly. These hypnozygotes were examined under the interference optics of a Zeiss Axiphoto microscope.

Surface sediments containing living cysts of *P. steinii* were collected from Omura Bay and Tokyo Bay with a TFO gravity corer in 1990. The top 2 cm of the sediments was cut and removed to a refrigerator below 4°C until starting the incubation experiment. The material was sonified and sieved with stainless screens of 120 μm and 20 μm in pore size, and the living cysts of *P. steinii* were picked up to inoculate in a culture dish with SW11 medium. The living cysts were cultured under 20°C, about 100 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ and 12 hours – 12 hours cycle. The culture was continued until one whole life cycle of this species from vegetative cells to planomeiocytes via planozygotes and hypnozygotes, was

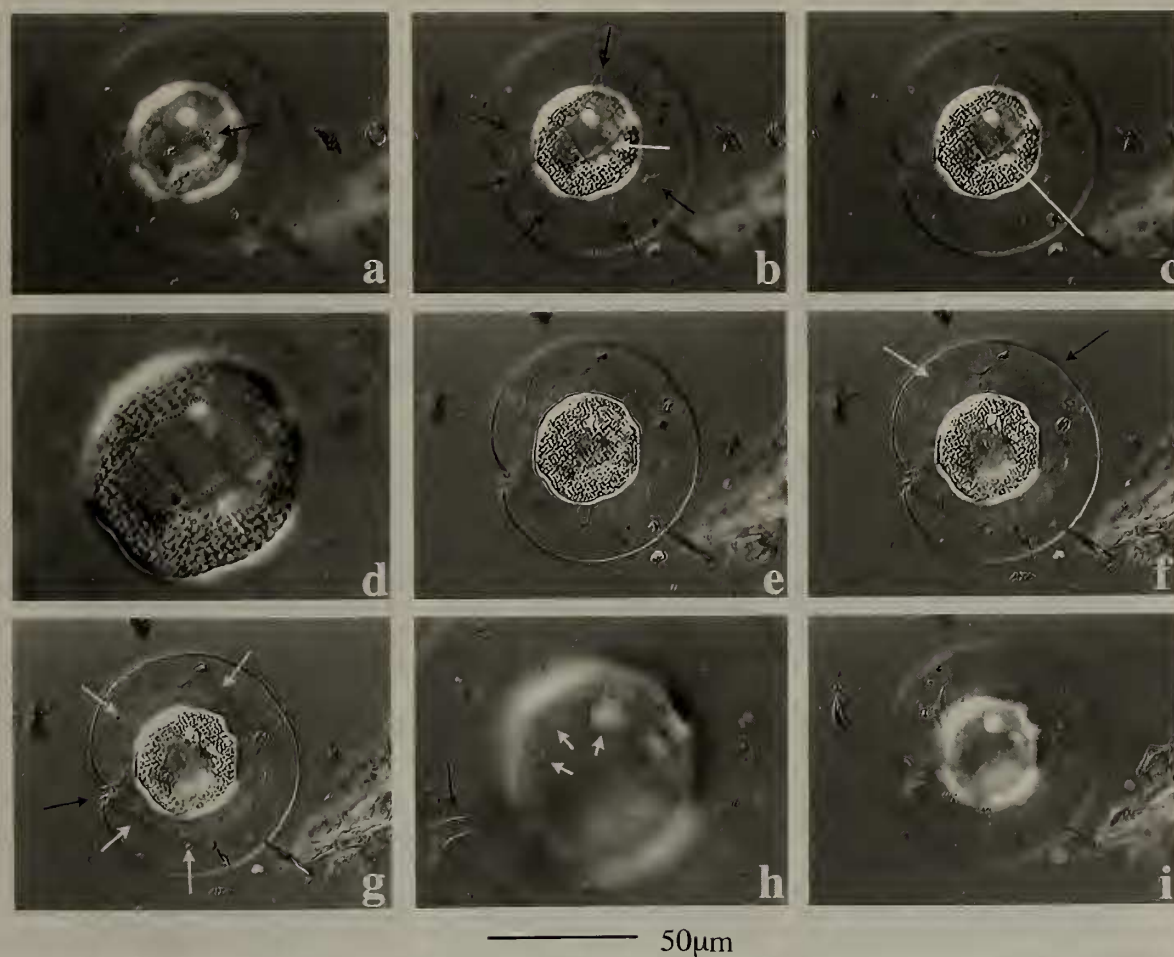


Figure 2. Hypnozygote involved by thecae of planozygote of *Pyrophacus steinii* in plankton sample collected from Omura Bay on June of 1990. A serial photographs from apical surface (a) to antapical surface (i). **a**; apical pore complex (black arrow) and 1' of the planozygote. **b** and **c**; three paraplates (?) corresponding to the archeopyle which has already appeared before germination of the hypnozygote (white arrows), and precingular processes (black arrows). **d**; enlargement of the archeopyle. **e**; optical cross section. **f**; hypotheca of planozygote (black arrow) and ectophragm of hypnozygote. **g**; sulcus of planozygote (black arrow) and postcingular processes of hypnozygote (white arrow). **h**; antapical processes distributing circularly (white arrows) and sulcus of planozygote (black arrow). **i**; antapical surface of planozygote.

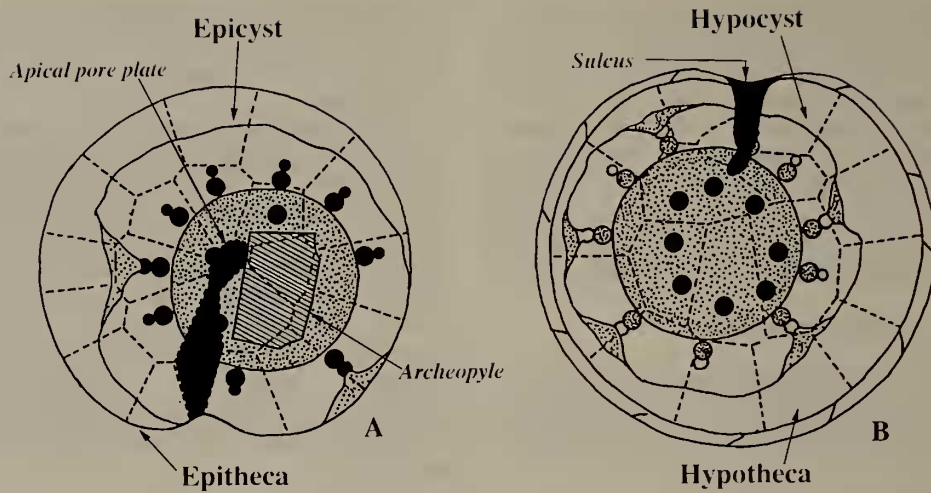


Figure 3. Illustration of a planozygote containing a hypnozygote, collected from Omura Bay, west Japan, June 1990. **A**; Epitheca and epicyst showing archeopyle sutures, **B**; Hypotheca and hypocyst, the same specimen of Figure 2.

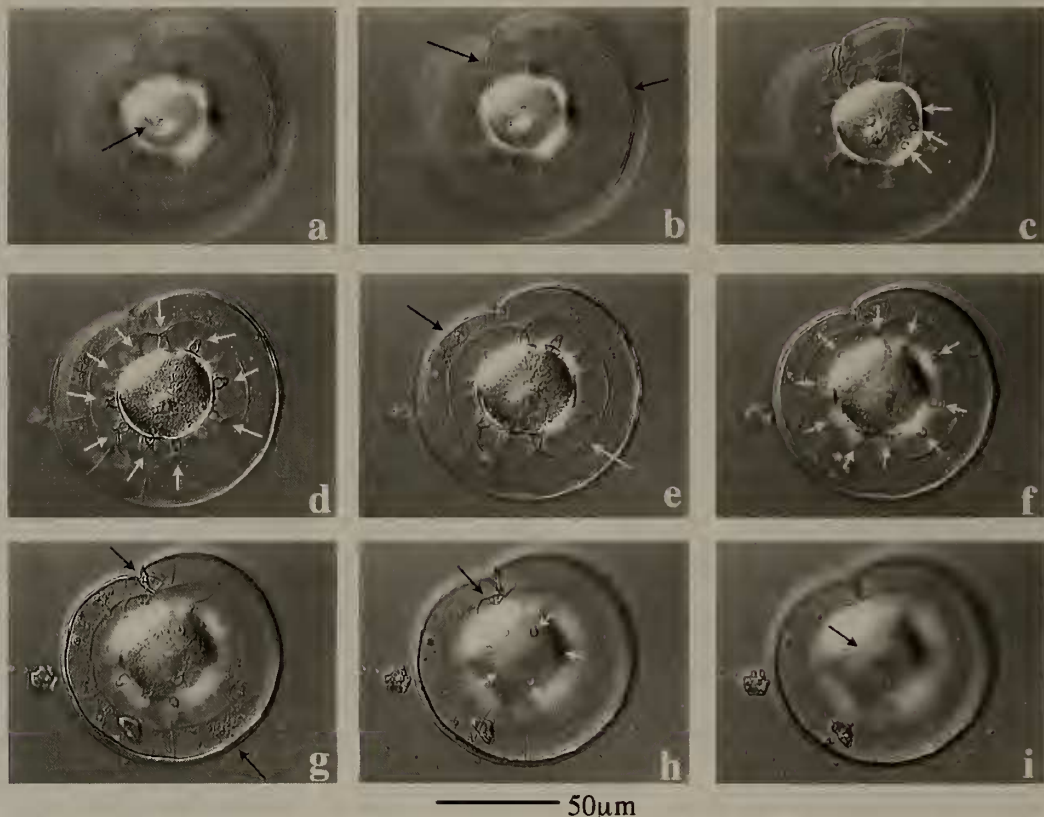


Figure 4. Hypnozygote involved by thecae of planozygote of *Pyrophacus steinii* in plankton sample collected from Omura Bay on June of 1990. Serial photographs from apical surface (a) to antapical surface (i). **a**; apical pore plate (black arrow). **b**; epitheca of planozygote and 1' plate (black arrows). **c**; apical processes with linear distribution (white arrows). **d**; precingular processes (white arrows). **e**; cingular plates of planozygote (black arrow) and ectophragm of hypnozygote (white arrow). **f**; postcingular processes (white arrows). **g**; sulcus of planozygote and hypotheca (black arrows). **h**; 3''' (black arrow) and antapical processes distributing circularly (white arrows). **i**; posterior intercalary of plate planozygote (black arrow).

completely observed. In particular, one specimen which had a well preserved archeopyle structure was carefully examined under interference optics of a Nikon Biophoto microscope.

Since the number of plates in *P. steinii* is (Figure 1) very variable, there have been several different interpretations of its expression. In this paper, we follow the interpretation of Matsuoka (1985).

Results

Plankton specimens of hypnozygotes (Figures 2, 3, 4)

A few immature resting cysts were collected in plankton samples from Omura Bay. These cysts were filled with fresh protoplasm and surrounded the planozygotic theca, clearly demonstrating a morphological relation between the planozygote and resting cyst. It is therefore easy to establish the orientation of the thecal plate and process distribution on the cyst.

The epitheca of the planozygote could easily be confirmed by the presence of the apical pore plate, seven apical, and eleven precingular plates (Figures 2, 3a). Beneath the apical pore plate, three large, barrel-shaped processes and a certain space were noticed on the cyst surface (Figures 2b, d). A polygonal outline similar to some plate boundaries confined this space. One row consisting of ten large and barrel-shaped processes ran along the shoulder (Figures 2b, 4d).

The opposite side of the hypnozygote contained the sulcus, and thirteen postcingular, three posterior intercalary, and three antapical plates were observed (Figures 2f, 3b). Beneath this hypotheca, eleven large barrel-shaped processes were formed on the shoulder of the cyst. Eight large barrel-shaped processes were circularly distributed on this cyst surface (Figures 2g, h).

The plate formula of the planozygote shown in Figure 2 was demonstrated as 7', 11'', 13c, 13''', 3p, 3'''' and 5s, while the process formula on the cyst would be described as 3' + 3@, 10'', 0c, 10''' and 8'''' (@ : paraplates consisting of the operculum).

Observation of empty hypnozygote surrounded by the planozygote (Figures 5, 6)

Some hypnozygotes used for our study on the life cycle remained within the planozygotic theca and its wall even after excystment (Figure 5). Figure 6 shows the morphological features of both planozygote and hypnozygote. The planozygote had one apical pore (Figure 5a), seven apical and eleven precingular plates. On the cyst surface just beneath this epitheca, there was an archeopyle probably corresponding to two paraplates (Figure 5c) and five larger barrel-shaped and three small, simple spherical processes distributed on one shoulder of the peripheral side (Figures 5d, e).

The hypotheca consisted of thirteen postcingular, three posterior intercalary, three antapical, thirteen cingular, and five sulcal plates (Figures 5j, k, l). On the cyst surface of this side, eleven large and barrel-shaped processes were located on the shoulder, and six large and barrel-shaped

processes positioned circularly (Figures 5g, h, i). The plate formula of this planozygote was 7', 11'', 13c, 13''', 4p, 1ap, and 3''', and the process formula of this cyst was 5 (3 + 2s)' + 2@, 11'', 0c, 10'', and 3'''' + 3s.

Observation on the transformation from planozygote to hypnozygote during incubation (Figure 7)

Clonal cultures of *P. steinii* were established by a single cell isolation from net-haul samples at Yokohama Umizuri-sambashi in innermost Tokyo Bay on June, 1990. During the clonal culture for this specimen, both sexual and asexual reproduction occurred. Sexual reproduction started to result in small and round thecate cells (male gametes). Female gametes were not morphologically differentiated from normal vegetative cells in plate formula and size. By sexual fusion of male and female gametes, a planozygote was produced and was morphologically similar to vegetative cells except for its larger cell size. After one to two days active swimming, the planozygote stopped moving, and sunk to the bottom of the culture chamber. The hypnozygotes were produced as a result of protoplasm contractions to approximately 1/5 of the original volume. After development of the exospore, many processes were formed between the exospore and the surface of the protoplast (mesospore). These processes were barrel-shaped and identical to those previously observed in fossil forms. This hypnozygote was contained within the theca of the planozygote (Figure 7). Several weeks after the encystment, a motile cell germinated from the hypnozygote through one opening (= archeopyle) formed on one flat side. As the theca of the planozygote was still attached to the surface of the hypnozygote, the position of the germinated side was easily determined with relation to the thecal plates. The archeopyle consisted of two paraplates, and above it, an apical pore plate with two furrows was observed (Figure 5c).

Discussion

Position and type of archeopyle

Wall (1967) established a new genus *Tuberculodinium* after revising *Pterospermopsis? vancampoe* Rossignol and emending the species diagnosis so that the archeopyle corresponds to a combination of precingular and intercalary paraplates. Drugg (1970) also considered the archeopyle of *Tuberculodinium* to be precingular with the number of detached opercular plates being variable in different species. Later, Wall and Dale (1971) observed the cysts of *P. steinii* and *P. vancampoe*, which now Balech (1979) and Matsuoka (1985) consider to be two subspecies of *P. steinii*, and noted that the archeopyle is hypocystal and that the number of paraplates comprising the operculum varies from two to four. However, this conclusion was not based on direct observation of germinated hypnozygotes, but on mostly theoretical grounds. The interpretation given by Wall and Dale (1971) was as follows: Four parallel rows consisting of large and barrel-shaped processes on the cysts were considered to represent apical, precingular, postcingular and antapical plate series of the thecate form respectively. Of these four rows, one contained two to five rectangular to polygonal



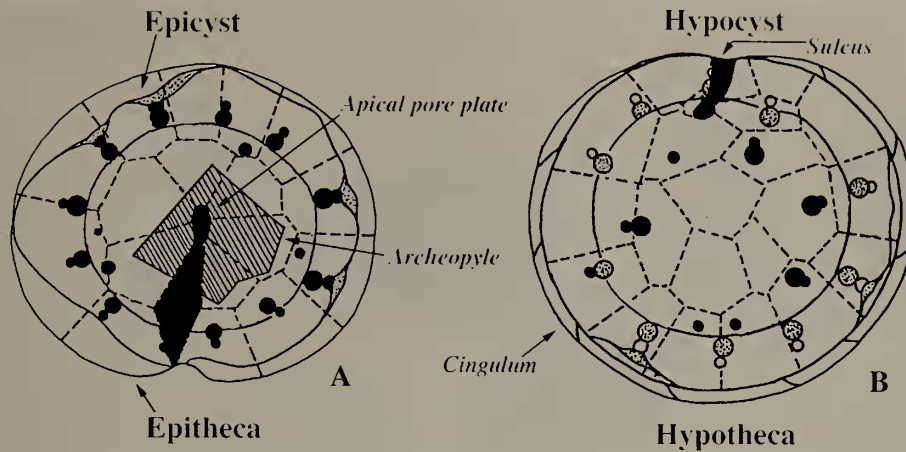


Figure 6. Illustration of a planozygote containing an empty hypnozygote after germination in culture. **A**; Epitheca and epicyst showing archeopyle sutures, **B**; Hypotheca and hypocyst, the same specimen of Figure 4.

paraplates forming the archeopyle which resembled posterior intercalary and sometimes additional posterior plates of the thecate cell. The processes opposite to this row were circularly distributed and apparently similar to the distribution of apical plate series of the vegetative cell. These observations led them to conclude that the cysts of *P. steinii* and *P. vancampoae* have a hypocystal archeopyle.

Matsuoka (1985) found hypnozygotes surrounded by the thecae of planozygotes in a plankton sample collected from Omura Bay. However, as the hypnozygotes were filled with protoplasm, he schematically illustrated the general outline of one specimen of the planozygote and hypnozygote using the same interpretation as Wall and Dale (1971).

Wrenn and Damassa (1989) examined hundreds of fossil specimens of *T. vancampoae* from latest middle Eocene to Holocene in detail, and concluded that the cyst morphology did not correspond to that of the theca; that the cyst was dorso-ventrally compressed and that the archeopyle was precingular rather than hypocystal. In order to rationalize this interpretation, Wrenn and Damassa (1989) proposed that during maturation of the planozygote, the hypnozygote rotated 90° with respect to the planozygote. A full description of this interpretation has yet to be published, and unfortunately we are therefore unable to evaluate their observations in detail. However, we never observed the hypnozygote to rotate from early to matured stage in the planozygote (Figure 7). According to our observations, we can also conclude that this is unlikely as suggested by Manum and Williams

(1995).

The specimens recovered from the net samples of Omura Bay also demonstrated archeopyle formation. These cysts, collected while still floating in surface waters before sinking, showed archeopyle structure on the hypnozygote surface even though these cells were filled with protoplasm. These archeopyles were not yet functional and the opercula were still in place, but the archeopyle suture could nevertheless be traced on the surface. The archeopyle was formed beneath the apical pore and apical plates of the planozygote. According to the outline of the sutures, the opercula seemed to be composed of three or four paraplates. However, none of the paraplates morphologically resembled any apical or precingular plates of the attached planozygote.

The incubated specimens clearly showed the position of an archeopyle, which was also formed beneath the apical pore plate and the apical plate series. In these specimens, although the opercula were completely detached leaving only their outline on the surface, the outline did not resemble either apical or precingular plates of the overlying planozygote. This observation agrees with the plankton specimens from Omura Bay.

Under these circumstances, the archeopyle of *T. vancampoae* (=hypnozygote of *P. steinii*) is formed on the epicyst, but it is very difficult to determine the type exactly. It should be called epicystal and compound rather than hypocystal or antapical.

Figure 5. Theca of planozygote and hypnozygote after germination. Serial photographs from apical view (a) to antapical view (l). **a**: apical pore plate and apical plates (3', 4', and 5'; black arrow). **b**: precingular plates (black arrows), **c**: large apical process (small white arrows) and archeopyle (large white arrow) formed on the surface of hypnozygote. **d**: large apical processes (white arrows) and endospore remaining in the cavity of the hypnozygote (black arrow). **e**: large precingular processes (black arrows). **f**: optical cross section of hypnozygote. **g**: cingular plates. **h**: large postcingular processes. **i**: 1''' (black arrow) and antapical processes distributed circularly (white arrows). **j**: 3''' (black arrow). **k**: posterior intercalary plates (black arrow). **l**: additional posterior intercalary plates (black arrow).

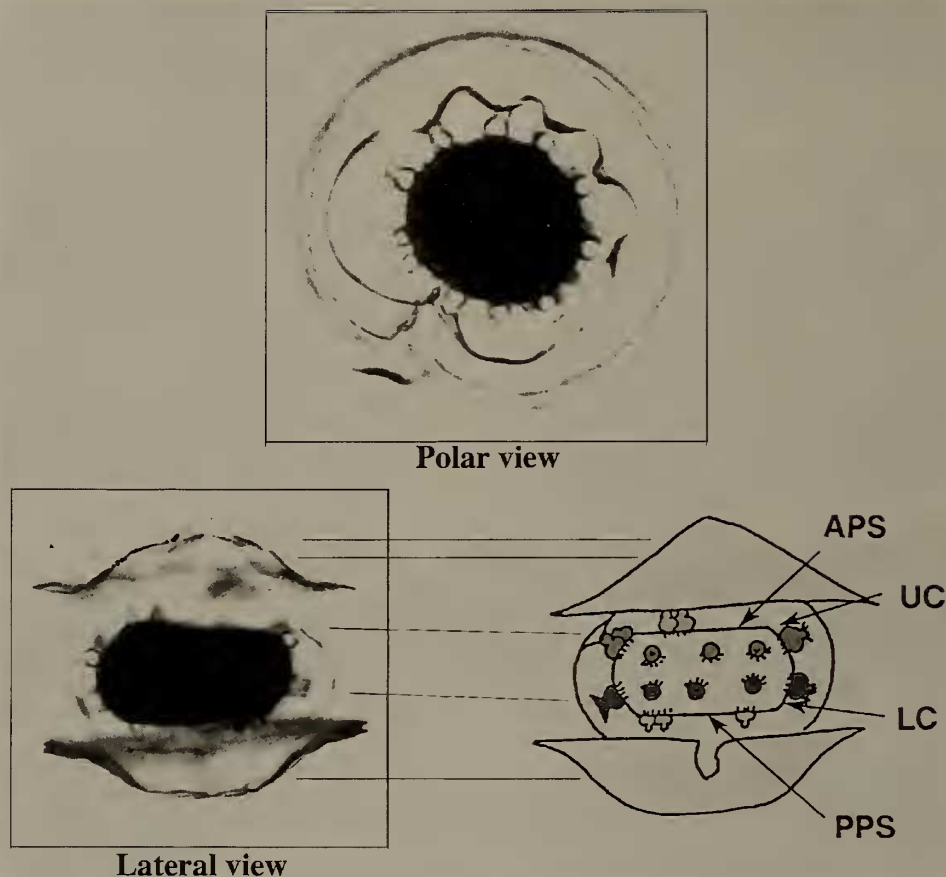


Figure 7. Hypnozygote produced within a planozygote of *Pyrophacus steinii* during the incubation experiment. APS : anterior flattened polar surface, UC : upper corner of flattened polar surface, LC : lower corner of flattened polar surface, PPS : posterior corner of flattened polar surface.

Intratabular structure on the hypnozygote

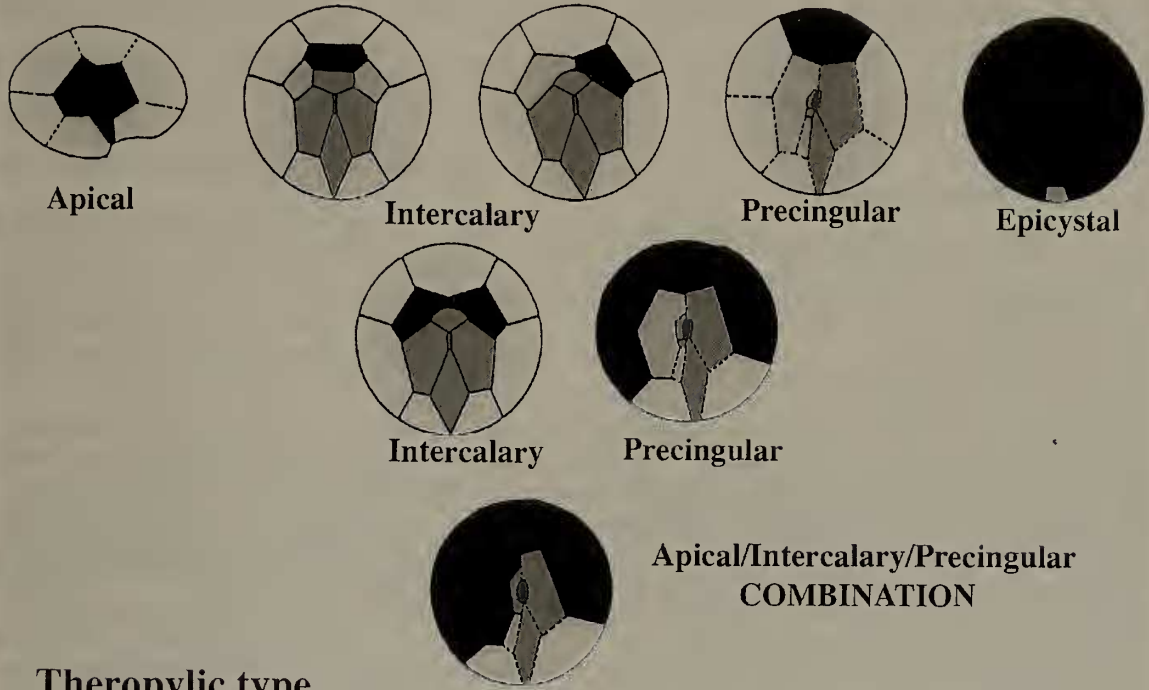
Stover and Evitt (1978) mentioned that intratabular features in *T. vancampoeae* are arranged in longitudinal rows of barrel-shaped processes and are absent in the equatorial area. Drugg (1970) considered intratabular features to be absent in two fossil species, *T. vancampoeae* and *T. rossignoliae*, and that except for the archeopyle, no parasutural features were observed. However, Wrenn and Damassa (1989) demonstrated an intratabular distribution as suggested by one barrel-shaped, large process placed in the center of one paraplate clearly bounded by parasutural features in fossil *Tuberculodinium vancampoeae*. The outline of these paraplates closely resembles each opercular piece.

According to Wall and Dale (1971) and Matsuoka (1985), the modern cysts of *Pyrophacus steinii*, including *P. steinii vancampoeae*, do not show any paraplate features like those in fossil specimens. Furthermore, there were no parasutures

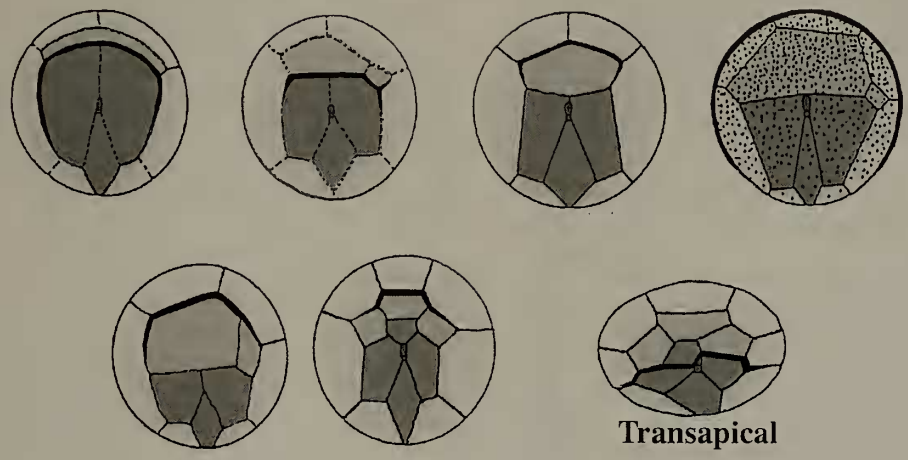
on the cyst and no paraplates except for archeopyle sutures which sometimes showed a polygonal outline. As with other gonyaulacoid cysts, the number of large, barrel-shaped processes is always fewer than that of thecal plates on both vegetative cells and planozygotes. There was no reflection of the cingulum, which was always shown by the space between two rows of large, barrel-shaped processes corresponding to pre- and postcingular plate series. The operculum detached from the cyst does not correspond with any apical and precingular plates of vegetative cells and planozygotes in shape and position.

For clear understanding how the processes are developed during maturation from the planozygote to hypnozygote, this progress should be continuously observed during incubation as was done on *Lingulodinium polyedrum* by Kokinos and Anderson (1995).

Saphopylic type



Theropylic type



Cryptopylic type

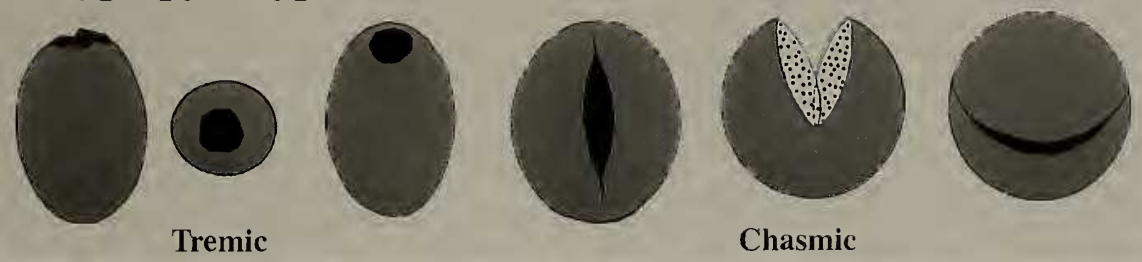


Figure 8. Archeopyle types developed in modern dinoflagellate cysts after modification of Matsuoka *et al.* (1989) based on the new observation.

Acknowledgments

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Reference cited

- Balech, E., 1979: El género *Pyrophacus steinii* Stein (Dinoflagellate). *Physis, sec. A*, 38, p. 27-38.
- Dale, B., 1978: Acritarchous cysts of *Peridinium faeroense* Paulsen: Implications for dinoflagellate systematics. *Palynology*, vol. 2, p. 187-193.
- Drugg, W.S., 1970: Two new Neogene species of *Tuberculodinium* and one of *Xenicodinium* (Pyrrhophyta). *Proceedings of the Biological Society of Washington*, vol. 83, p. 115-122.
- Evitt, W.R., 1963: A discussion and proposals concerning fossil dinoflagellates, hystrichospheres, and acritarchs, I, II. *Proceedings of the Natural Academy of Science*, vol. 49, p. 158-164 and p. 298-302.
- Evitt, W.R., 1985: *Sporopollenin Dinoflagellate Cysts-Their morphology and interpretation*, xv+333 p. American Association of Stratigraphic Palynologists Foundation, Texas.
- Harding, I.C. and Lewis, J., 1994: Siliceous dinoflagellate thecal fossils from the Eocene of Barbados. *Palaeontology*, vol. 37, p. 825-840.
- Kokinos, J.P. and Anderson, D.M., 1995: Morphological development of resting cysts in cultures of the marine dinoflagellate *Lingulodinium polyedrum* (= *L. machaerophorum*). *Palynology*, vol. 19, p. 143-166.
- Lentin, J.K. and Williams, G.L., 1993: *Fossil dinoflagellates: Index to genera and species 1993 edition*. AASP Contribution Series No. 28, viii+856 p. American Association of Stratigraphic Palynologists Foundation.
- Manum, S. and Williams, G.L., 1995: Hypocystal archeopyles in the dinoflagellate cyst genus *Calligodinium* Drugg. *Palynology*, vol. 19, p. 183-190.
- Matsuoka, K., 1985: Cyst and thecate forms of *Pyrophacus steinii* (Schiller) Wall et Dale. *Transactions and Proceedings of the Palaeontological Society of Japan, New Series*, 140, p. 240-262.
- Matsuoka, K., 1988: Cyst-theca relationships in the diplosalid group (Peridiniales, Dinophyceae). *Review of Palaeobotany and Palynology*, vol. 56, p. 95-122.
- Matsuoka, K., Fukuyo, Y., and Anderson, D.M., 1989: Methods for modern dinoflagellate cyst studies. In: Okaichi, T., Anderson, D.M., and Nemoto, T. eds., *Red Tides: Biology, Environmental Science, and Toxicology*, p. 461-479. Elsevier, New York.
- Rosignol, M., 1962: Analyse pollinique de sédiments Quaternaires en Israël. I: Sédiments Pleistocènes. *Pollen et Spores*, vol. 4, p. 121-148.
- Stover, L.E. and Evitt, W.R., 1978: *Analyses of Pre-Pleistocene organic-walled dinoflagellates*. Stanford University Publications, Geological Sciences, 25, iii+300 p., Stanford, California.
- Wall, D., 1967: Fossil microplankton in deep-sea cores from the Caribbean Sea. *Palaeontology*, vol. 10, p. 95-123.
- Wall, D. and Dale, B., 1971: A reconsideration of living and fossil *Pyrophacus* Stein, 1883 (Dinophyceae). *Journal of Phycology*, vol. 7, p. 221-235.
- Wrenn, J.H. and Damassa, S.P., 1989: *Tuberculodinium vancampoae*: A curious reflection of its former self. *Abstract of Fourth International Conference on Modern and Fossil Dinoflagellates*, p. 108, Woods Hole, Massachusetts, U.S.A.